Department of Infectious & Parasitic Disease Pathology

Douglas J. Wear, M.D.
Chairperson
Date of Appointment - 27 June 1988

ORGANIZATION

The department is organized into four divisions and the Office of the Chairperson.

Division of AIDS Pathology - Ann Nelson, M.D.

Division of Microbiology - Ted L. Hadfield, LtCol, USAF, BSC

Division of Geographic Pathology - Douglas J. Wear, M.D.

Division of Molecular Pathobiology - Shyh-Ching Lo, M.D., Ph.D.

STAFF

Office of the Chairperson

Medical

Douglas J. Wear, M.D.

Administrative

Darlene Wilson, Secretary to the Chairperson

CONSULTATION

Casess

Description	*Reported*
Military	156
Federal(VA/PHS)	93
Civilian	796
Interdepartmental	1,891

RESEARCH

See individual division reports.

EDUCATION

The department provided training to three individuals. In addition, three high school students spent the summer in the department performing research under the direction of staff members.

GOALS

Education

1. Continue producing learning sets (2X2 transparencies) by organ systems as a complement to the adult and pediatric AIDS fascicles (completed).

2. Expand in quantity these learning sets (2X2 transparencies) by organ systems in preparation for sale through the ARP.
3. Continue developing learning sets (glass slides) by organ system as a complement to the adult and pediatric AIDS fascicles.
4. Begin the monographs on infectious AIDS pathology entitled *Pathology of AIDS-related Infectious Diseases in Adolescents and Adults* and finish the monograph *Pathology of AIDS in Children* (completed).
5. Prepare a new edition of *Pathology of Tropical and Extraordinary Diseases*, an atlas entitled *Pathology of Infectious Disease.*
6. Prepare audiovisual material for teaching using the latest computer-generated programs, videotape, and CD-ROM techniques.
7. Present the 8th Infectious Disease Course, "Emerging Infections: Clinical and Pathologic Update."
8. Assist the Museum in the development of a pilot exhibit on emerging infections.
9. Continue lectures to regional elementary and high schools on infectious diseases and AIDS.
10. Provide lectures for the new Triservice Tropical and Infectious Disease Course.
11. Provide educational support for government agencies such as USUHS, the Coast Guard, the Peace Corp, and NIH and nongovernment agencies and societies.

Research

1. Continue a histopathologic study of bartonella infections in cat-scratch disease (CSD) in both immunocompetent (CSD) and immunoincompetent bacillary angiomatosis patients.
2. Finalize characterization of the genome of the AIDS-related mycoplasma, *Mycoplasma fermentans* (incognitus strain), and characterize genome of newly found *Mycoplasma fermentans.*
3. Continue to isolate the AIDS-associated mycoplasma, *M fermentans* (incognitus strain), *M penetrans,* and other emerging human mycoplasmas from blood, urine, and biopsy sites of infected patients.
4. Finalize studies to establish a serological service laboratory to test patient material for mycoplasma.
5. Study various mycoplasma infections in veterans participating in Desert Storm.
6. Continue to search for previously unidentified microorganisms in patients with AIDS and to study their possible pathogenicity.
7. Explore the possible role of chronic infection with mycoplasma in the pathogenesis of various human diseases.
8. Employ polymerase chain reaction (PCR) tests to identify cat-scratch bacilli in culture and tissues and continue to develop PCR tests against additional infectious agents, particularly those associated with immunosuppression.
10. Study the pathogenesis of *Mycobacterium ulcerans* infections (Buruli ulcer) with a view to the development of a vaccine for immunoprophylaxis of the disease: laboratory and field study.
11. Determine the prevalence of naturally acquired leprosy and SIV in nonhuman primates in West Africa.
12. Develop the experimental research station in West Africa on experimental leprosy in nonhuman primates.

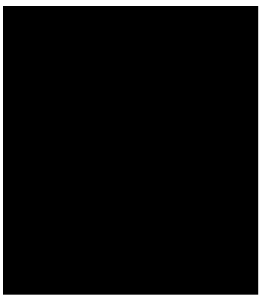
13. Continue to do fatty acid profiles of difficult-to-identify bacteria through gas chromatography identification of cell wall lipids.
14. Support initiatives to obtain funds to establish adequate facilities to support applied and basic research on HIV infection.
15. Continue studies for brucella vaccine development.
16. Continue lipid analysis of *Yersinia pestis* and *Francella* spp for rapid detection.
17. Continue animal efficacy studies on brucella vaccine project.
18. Isolate and characterize the cause of infectious ferret diarrhea.
19. Continue the study of lymphoma and solid tumors in AIDS patients.
20. Continue the study on the role of dentritic cells (antigen presenting) in the pathogenesis of HIV infection and complications.
21. Explore methods for identification of bacteria using mass spectroscopy.

PRESENTATIONS

See individual division reports.

PUBLICATIONS

See individual division reports.



DIVISION OF ACQUIRED IMMUNODEFICIENCY SYNDROME (AIDS) PATHOLOGY & EMERGING INFECTIOUS DISEASES

Ann M. Nelson, M.D.
Chief
Date of Appointment - 1 March 1995

MISSION

The basic charter of the AIDS Pathology Division and Registry is to determine the pathology of AIDS through the study of all the pathological changes in the human body resulting from infection with the human immunodeficiency virus (HIV), any possible cofactors, associated opportunistic infections, and unusual neoplasias. The division provides consultative services to federal (military and civilian), nonfederal, and foreign (military and civilian) institutions. International cooperative projects for research and education are in progress, and new ones are encouraged.

STAFF

Diagnostic Histopathology

Medical

Ann M. Nelson, M.D., Chief (VA)
Sarah G. Frankel, M.D., Staff Pathologist (ARP)
(D) Choukria Mered, M.D., AIDS Researcher (ARP)
(A) Mary Klassen, M.D., Staff Pathologist (on loan from Pulmonary & Mediastinal Pathology)

Technical Support

Blair B. Slaughter, Histology Technician (ARP)
Mildred D. Benton, Histology Technician (ARP)
Jacqueline Martinez, Research Clerical Technician (ARP)
Albin Moroz, Computer Programmer (ARP)

Administrative Support

(D) Patricia Lissner, AIDS Fascicles Editor (ARP)
Cynthia Wilson, Secretary (ARP)

CONSULTATION

Cases

Description	Received
Military	21
Federal	55
Civilian	278
Intramural	65

We received 363 cases and reviewed 65 intramural consults, for a total of 428 cases studied. A preliminary report is sent by fax to the contributor, usually within 24 hours of receipt of the case; additional clinical information is requested with the fax. More than 75% of contributors respond with the requested information. The final reports provide information on the Centers for Disease Control and Prevention classification system for HIV disease, pertinent bibliography, copies of special staining techniques, representative glass slides, and representative 2x2 transparencies (as appropriate). The AIDS Division staff supported the other elements of the department by attending diagnostic conferences and by providing analysis and consultation in the diagnosis of other cases.

All cases and consults on HIV-seropositive patients are entered in the AIDS data base. As of 31 December 1995, a cumulative total of 5,066 cases had been accessioned into the AIDS data base system, approximately 25% are autopsy specimens.

RESEARCH

The basic research charter of the division and the Registry of AIDS Pathology is to collect, catalogue, and prepare for multilayered scientific study as many cases of AIDS and AIDS-related conditions as possible. Research projects are developed using the data base and material in the AIDS Registry.

1. The project entitled "Computer Survey of the AFIP Repository for Cases of Acquired Immunodeficiency Preceding the HIV Pandemic" (UBGA), supported by AFIP/USA MRDC, successfully completed its second phase of planned objectives: To seek, review, identify, and retrieve repository materials (slides, blocks, wet tissues, and information) of cases fulfilling the CDC definition of AIDS in the absence of demonstrable HIV infection. To identify cases for potential

use in basic research on the chronology of HIV retroviral infection in human tissues. A complete computer network was implemented at the AIDS Pathology Division that maximizes the efficiency of interactions between all investigators. An AIDS data base system has been developed that allows tracking of AIDS cases, both prospectively and retrospectively. The Structured Query Language (SQL) capabilities allow easy access to the downloaded information from the AFIP mainframe based on multiple variables or combinations of variables. The new program is also capable of doing comparisons of data from the pre-AIDS era with cases in the AIDS Registry. Merge documents, graphics, and other presentation formats can be generated directly from the data base.

2. The project entitled "AIDS-related Kaposi Sarcoma in Women: The Search for the AIDS-related Kaposi Sarcoma Cofactor" (UBSE) is in progress. Principal investigator: Kathleen Smith, M.D., dermatopathologist, visiting, AIDS Registry; dermatologist. The objectives are to identify women with Kaposi sarcoma and (1) describe the clinical history and histology of such cases; (2) evaluate the sexual and drug-abuse histories; (3) compare and contrast the clinical histories and histologic features of AIDS-related Kaposi sarcoma in women to those in non-AIDS-related Kaposi sarcoma in women; and (4) evaluate whether the amount of nitrite use correlates with the timing of Kaposi sarcoma development among AIDS patients.
3. The project entitled "Dendritic Cells and HIV Infection: A Series of Tonsil and Adenoid Tissues" (collected in the Division of Otolaryngic Pathology) was studied. The quantity and distribution of dendritic, lymphoid, and epithelial cells were profiled using immunohistochemical methods. In addition, immunohistochemistry (for P24 gag protein) and in situ hybridization were performed to localize and semiquantify HIV. Data indicate that the lymphoepithelium is a site of productive infection. Future plans include expanded review of lymphoepithelia in these and other sites.

Several small projects are being done on various complications of HIV infection and AIDS. These include solid tumors, parvovirus B19 infection and anemia, and unusual opportunistic infections.

EDUCATION

The division contributed to its educational mission by: (1) participating in daily departmental slide conferences for review of unique, controversial, difficult, or interesting cases; (2) presenting lectures and unknown glass slide seminars at the AFIP weekly professional staff conferences; (3) attending seminars at regional, national, and international meetings; and (4) presenting lectures, posters, and seminars at local, regional, national, and international meetings.

Material accessioned under the AIDS data base system continues to be used in the preparation of AIDS pathology study sets and of medical articles to be submitted to peer-reviewed specialty journals. Up to the present, 160 sets of slides have been cut, stained, and catalogued. Glass slides and color transparencies have been collected and are being added to the master AIDS data base, which eventually will be used by staff at the AFIP through the integrated LAN computer system. Material has been provided to the Division of Gastrointestinal Pathology and the Department of Hematologic and Lymphatic Pathology for preparation of study sets.

Draft completion of a fascicle of pediatric AIDS pathology was completed, and another of adult AIDS infectious pathology is projected for early 1997. Special emphasis is being placed on the practical diagnosis of remarkable conditions presenting to general pathologists in the civilian and military communities.

No course was held in 1995 on infectious disease and AIDS pathology. A course on emerging infectious diseases is planned for April 1996, in Atlanta, in collaboration with Emory University and the Centers for Disease Control and Prevention Services. The codirectors are Dr. Nelson and Dr. Robert Horsburgh (Emory University).

The *Synopsis of AIDS Pathology in Children* was essentially completed in 1994. All chapters have

been submitted to Dr. Florabel G. Mullick, SES, by the scientific editor, Dr. Cesar Moran. Work on the *Synopsis of AIDS Pathology in Adults* will begin in 1996.

RESENTATIONS

Sarah S. Frankel, M.D.

Community Education

AIDS education, 8th grade, Charles E. Smith Jewish Day School (JDS), February 9.

AIDS education, 6th grade, JDS, February 27.

Judge at Takoma Park School Science Fair, March 9.

AIDS education, high school students, AFIP, May 19.

"HIV and the Immune System," DOD Summer Apprentice Program, June 21.

"Living in a World With AIDS," NMHM, August 26.

AIDS education, 7th grade, Takoma Park School, November 8.

AIDS education, 7th grade, Charles E. Smith Jewish Day School (JDS), December 6.

Docent training, NMHM, December 14.

Other

"Complications of HIV Infection in Women: A Pathologist's Perspective," HIV Infection in Women, February 23.

"The Altered Histology of Infectious Disease Pathology in AIDS and Other Immunocompromised Hosts," Problems in Anatomic Pathology, AFIP, April 11.

Weekly Hematology-Hematopathology Conference, Georgetown University Medical Center, May 8.

"AIDS and Universal Precautions," the Triservice School of Histotechnology, May 26.

"The Altered Histology of Infection in the HIV Infected Patient," the AOA-AFIP Biennial Meeting, Leavey Conference Center, June 23.

"Introduction to the Histology of the Head and Neck," Georgetown University Medical Center, Department of Pathology Residents Conference, July 10.

Case presentations for the ARP Senior Resident Symposium, AFIP, September 15.

"Microscopic Histopathology of AIDS," Resident Slide Conference, Georgetown University Medical Center Department of Pathology, September 9.

"Dendritic Cells in HIV Infection," WRAIR, MMCAR, Rockville, October 13.

"Genetics," Georgetown University School of Medicine, Sophomore Pathology Course, October 20.

"Microscopic Histopathology of AIDS Lymph Nodes," Resident Slide Conference, Georgetown University Medical Center Department of Pathology, November 30.

"AIDS and the Immune System," Georgetown University School of Medicine, Sophomore Pathology Course, November 13.

"Dendritic Cells in HIV Infection," Veterinary Pathology Conference, AFIP, December 1.

Dr. Nelson:

"Pathology of Tuberculosis and MAI Infection," Walter Reed Pathology Department, February 16.

Binford-Dammin Society Scientific Seminar, organizer and moderator, March 11.

"The Pathology of Infectious Disease and AIDS," Osler course, lecture and handout, April 6.

Overview of the Division of AIDS Pathology, AFIP, briefing to the Deputy Surgeon General of the Royal Thai Army, April 21.

Briefing of Russian pathologists from Duke University, overview of AIDS Pathology, May 16.

NIH Unknown Slide Conference, pathology residents, May 17.

"Radiologic-Pathologic Correlation of Gastrointestinal Manifestations of AIDS," AFIP Wednesday Staff Conference, June.

"The Pathology of HIV Infection and AIDS (I, II,III)," AFIP course, Controversies and Recent Advances in Surgical Pathology, Snowmass, Colorado, July.

"HIV Infection and AIDS," Medical Student Symposium, Autonomous University of Guadalajara, Guadalara, Mexico, October.

Keynote address at Premedical Advisors Meeting, Autonomous University of Guadalajara, Guadalara, Mexico, October.

"The Pathology of HIV Infection and AIDS," Medical Staff Conference, Autonomous University of Guadalajara, Guadalajara, Mexico, October.

"History of Emerging Infections at the Army Medical Museum and AFIP," docent training, NMHM, November.

PUBLICATIONS

1. Pantongrag-Brown L, Nelson AM, Brown AE, Buetow PC, Buck JL. Gastrointestinal manifestations of acquired immunodeficiency syndrome: radiologic-pathologic correlation. *Radiographics*. 1995;15:1155-1178.
2. Moran CA, Nelson AM, Tuur SM, Luengo M, Fonseca L, Meyers WM. Leprosy in five human immunodeficiency virus-infected patients. *Mod Pathol*. 1995;8:662-664.
3. Sifuentes-Osorino J, Ponce-de-Leon LA, Camacho-Mezquita FE, Bobadillo-del-Valle JM, Infante-Suarez ML, Rameriz-Fernandez N, Hernandez-Gomez L, Nelson AM. Resistencia de *Mycobacterium tuberculosis* en pacientes mexicanos. *Rev Invest Clin*. 1995;47:273-281.

Dr. Frankel:

Grants

Antigen Presenting Cells in Human Immunodeficiency Virus Infection and Acquired Immunodeficiency Syndrome, American Registry of Pathology, October 1, 1995 to September 30, 1996.

Preceptorships

Preceptor, Department of Defense Science and Engineering Apprentice Program, June 19 to August 11, 1995. Natae Nash. Discrepancies Based on Gender in AFIP Accessions.

Summer student preceptor, AFIP/ARP, June 20 to August 17, 1995. Lesley Steinman. Developed teacher's guide for NMHM exhibit "Living in a World With AIDS."

DIVISION OF MICROBIOLOGY

Ted L. Hadfield, LtCol, USAF, BSC
Chief
Date of Appointment - April 1989

MISSION

The Division of Microbiology performs research and provides case consultations to support the Department of Infectious and Parasitic Disease Pathology in meeting its missions of consultation, research, and education.

ORGANIZATION

The Division is divided into two branches.

The Mycobacteriology Branch - Wayne Meyers, M.D., Ph.D.

The Bacteriology Branch - Ed Hilyard, LT, MSC, USN

STAFF

Medical

Wayne M. Meyers, M.D., Ph.D.

Scientific

Bob Crawford, Ph.D. Senior Scientist/Immunologist
Susan Elizabeth Drazek, Ph.D., Microbiologist
Ed Hilyard, LT, MSC, USN, Scientist
Ellen D'Nicuola, B.S., Medical Technologist
Bertha L. Evans, HM2, USN, Division NCO
(A) Claxton Whiteside, USA, Medical Technician
Craig Hammock, Scientist, Flow Cytometry Support
Wendy Goodman, Editorial Assistant
(A) Estel Page, Laboratory Worker, Glassware
William Cunningham, Laboratory Worker, Glassware
(D) Lillie Harris, Laboratory Worker, Glassware
(D) John McGraw, SPC, USA, Laboratory Technician

CONSULTATION

Cases

Description	*Reported*
Military	4
Federal VA/PHS	2
Intramural	11
Civilian	217

Microbiology staff supported the other elements of the Department of Infectious and Parasitic Disease Pathology by performing biochemical analyses and molecular biology analyses and by providing consultation. Additional support to the Institute staff includes sterilization facilities for infectious waste, quality testing of laboratory water, synthesis of oligonucleotides for research and diagnosis, and operation of the central glassware facilities to provide clean and sterile glassware for the staff at AFIP.

EDUCATION

The staff presented 26 lectures, published 13 research papers, and attended 9 national and international meetings during 1995.

The staff participated in two courses sponsored by the Department of Infectious and Parasitic Disease Pathology, held at AFIP and Raddison Park Terrace Hotel. Staff personnel were invited chairpersons to two symposia, officers to three organizations, or invited representatives to two international organizations. Wayne M. Meyers, M.D., Ph.D., was selected civilian employee of the year and Edward J. Hilyard, LT, MSC, USN, received a "Best Poster" award at the annual Society of Armed Forces Medical Laboratory Scientists meeting, in San Antonio.

RESEARCH

The staff conducted research on the following topics during the year: experimental leprosy in mangabey, rhesus, cynomolgus, and African green monkeys; leprosy vaccine testing in mangabey monkeys; AIDS in leprosy patients; histopathology of early lesions of leprosy; histopathologic changes of leprosy lesions following multidrug therapy; naturally acquired leprosy in wild animals; Buruli ulcer (*Mycobacterium ulcerans* infections) in West Africa; lymphadenitis caused by a nonculturable, nonleprosy mycobacterium; cytotoxic CD8+ cells (TAI-1) in cellular exudates in leprosy; experimental leprosy in SCID mice; development of a DNA probe and an ELISA assay for cat-scratch disease bacilli; use of fatty acid methyl esters for characterization and/or identification of bacteria and mycobacteria; fatty acid characterization of unusual bacteria; application of *Mycobacterium tuberculosis* and *Mycobacterium avium* DNA probes in polymerase chain reactions; development of polymerase chain reaction for detection of bacteria from paraffin-embedded tissues and DNA sequencing for identification of organisms; and development of a *Brucella melitensis* component vaccine and live attenuated *Brucella melitensis* vaccine for use in humans.

GOALS

The goals of the Division of Microbiology are: to understand leprosy and Buruli ulcer, i.e., to establish the importance of nonhuman reservoirs of *Mycobacterium leprae* in the epidemiology of leprosy in humans; to develop newer methods of treatment and control of Buruli ulcer, including chemotherapy and vaccination; to develop and implement molecular genetic assays to complement basic histopathology diagnosis for consultations; and to develop a vaccine for *Brucella melitensis*. We also want to promote an understanding and means for diagnosis of *Bartonella henselae* infection and its role in cat-scratch disease and bacillary angiomatosis and to develop a comprehensive understanding of immunologic responses to *Brucella melitensis,* allowing development of a component and live vaccine for *Brucella melitensis*. An additional goal of the division is to complete the bacteriology portion of the *Atlas for Infectious Diseases*.

PRESENTATIONS

1. Hadfield TL. Establishment of FAME data base and two-dimensional analysis of library and test organisms for CBMS, presented at Colorado School of Mines, Department of Chemistry and Geochemistry February 9, 25 people in attendance.
2. Hadfield TL. Brucellosis, AFIP Staff Conference, April 5, 50 people present.
3. Hadfield TL. Brucella Vaccine Development, Review for the Deputy Surgeon General, Royal

Thai Army, April 21.

4. Hadfield TL. Brucella Safety, review for animal technicians, Laboratory Animal Medicine staff, AFIP, 30 minutes, April 25.
5. Hadfield TL. Brucellosis and Brucella Vaccine Development, Laboratory Animal Medicine, AFIP, 10 people, 1 hour.
6. Bhattacharjee AK, Hadfield TL, Taylor R, Zollinger WD, Van de Verg L, Warren RL, Yuan L, Hoover DL. Immunogenicity of *Brucella abortus* and *Brucella melitensis* Lipopolysaccharides in Mice and Optimization of Vaccine Formulations and Regimen, National Meeting of the American Chemical Society, August 20-25.
7. Hadfield TL. Current Investigations in the Division of Microbiology, Huntington Conference Room, November 30, Museum Curators Group.
8. Basile Franco, Hadfield TL, Sickenberger DW, Voorhess KJ. Comparison of Microorganism Identification/Differentiation Methods Based on Fatty Acid Methyl Ester Analysis: Gas Chromatography and Pyrolysis/Mass Spectrometry, Analytical Chemistry in Environmental Microbiology, March 12-16, abstract.
9. Basile F, Hadfield TL, Sickenberger DW, Voorhees, KJ. PY/MS FAME Extract Profiling of Pathogenic Microorganisms, American Society Mass Spectrometry, May, abstract.
10. Hadfield, TL, Brockett R, Fisher DR, Trevino S, Willett C, Atamosa C, Walker N, Garcia M, Astorga A, Dolan D, Banfield S, Gonzalez C, Tapia R, Libby A. Case studies in Microbiology Workshop, Society of Armed Forces Medical Laboratory Scientists 24th Annual Meeting, March 4, 75 participants, 4 hours.
11. Battacharjee AL, Hadfield TL, Taylor R, Zollinger WD, Van De Verg L, Warren RL, Yuan L, Hoover DL. Immunogenicity of *Brucella Abortus* and *Brucella melitensis* Lipopolysaccharides in Mice and Optimization of Vaccine Formulations and Regimen, American Chemical Society, abstract.
12. Hilyard EJ, Hadfield TL, Smith K, Skelton H, Moeller R, Tuur S, Decker C, Wagner K, Angritt P. Identification of Tyzzer's Bacillus in Humans, SAFMLS, March 3, poster.
13. Hilyard EJ. Chemical Biological Mass Spectrometer, Huntington Conference Room, November 30, Museum Curators Group.
14. Drazek S, Crawford R, Hadfield TL, Hoover DL, Warren RL. Deletion of purE Attenuates *Brucella melitensis* for Growth in Human Monocyte-Derived Macrophages, AFIP Staff Conference, April.
15. Crawford R, Hadfield TL, Warren R, Drazek S, Houng H, Van De Verg L, Hoover DL. Deletion of purE Attenuates *Brucella melitensis* Infection in Mice, ASM, May, abstract.
16. Crawford R, Hadfield TL, Warren R, Drazek S, Van De Verg L, Hoover D. Development of Murine Model for *Brucella melitensis* Infection, Brucellosis Research Conference, November, abstract.
17. Crawford R. Bacterial Growth and Host Responses in Mouse Model of Brucellosis Infection, AFIP Staff Conference, April.
18. Crawford R. Macrophage Activation and Antimicrobial Effector Function, Catholic University, March.
19. Meyers WM. Buruli Ulcer in Togo: First Reported Patients, Weekly Professional Staff Conference, Armed Forces Institute of Pathology, Washington, D.C., April.
20. Meyers WM. Tropical Dermatology, lecture, Peace Corps Officers Staff Development Program, Peace Corps Headquarters, Washington, D.C., May.

21. Gormus BJ, Meyers WM, et al. Protective Vaccination of Monkeys Against Leprosy by BCG or BCG Plus Heat-killed *Mycobacterium leprae*, American Society for Microbiology, Washington, D.C., May.

22. Meyers WM, Neafie RC, Marty AM. Onchocerciasis, Streptocerciasis, Loiasis and Buruli Ulcer, Tropical Medicine Course, Walter Reed Army Institute of Research, Washington, D.C., July.

23. Meyers WM. Leprosy, Tropical Medicine Course, Walter Reed Army Institute of Research, Washington, D.C., July.

24. Meyers WM. Pathogenesis and Pathology of Buruli Ulcer, European Conference on Tropical Medicine, Hamburg, Germany, October.

25. Portaels F, Fonteyne PA, De Beenhouwer H, de Rijk P, Guedenon A, Hayman J, Meyers WM. Variability in the 3' end of the 16S rRNA Sequence of the Species *Mycobacterium ulcerans* Is Related to Geographic Origin of Isolates, European Conference on Tropical Medicine, Hamburg, Germany, October.

26. Meyers WM. Histopathologic Diagnosis of Some Extraordinary and Perplexing Diseases of the Tropics, Symposium: Contemporary Pathology in Tropical Medicine, cochaired by Paul Racz and W. M. Meyers, European Conference on Tropical Medicine, Hamburg, Germany, October.

27. Meyers WM. Three-part lecture: Leprosy in Nonhuman Primates, *Mycobacterium ulcerans* Infections, Leprosy in SCID Mice, Lew Institute for Biomedical Research, Seoul, Korea, November.

PUBLICATIONS

1. Breitschwerdt EB, Kordick DL, Malarkey DE, Keene B, Hadfield TL, Wilson K. Endocarditis in a dog due to infection with a novel *Bartonella* subspecies. *J Clin Microbiol*. 1995;33:154-160.

2. Basile F, Voorhees KJ, Hadfield TL. Microorganism Gram-type differentiation based on pyrolysis-mass spectrometry of bacterial fatty acid methyl ester extracts. *Appl Environ Microbiol*. 1995;61:1534-1539.

3. Drazek ES, Crawford R, Hadfield TL, Hoover D, Warren RL. Deletion of purE attenuates *Brucella melitensis* 16M for growth in human monocyte-derived macrophages. *Infect Immun*. 1995;63:3297-3301.

4. Kordick DL, Wilson KH, Sexton DJ, Hadfield TL, Berkhoff HA, Breitschwerdt EB. Prolonged *Bartonella* bacteremia in cats associated with cat-scratch disease patients. *J Clin Microbiol*. 1995;33:3245-3251.

5. Converse PJ, Haines VL, Wondimu A, Craig LE, Meyers WM. Infection of SCID mice with *Mycobacterium leprae* and control with antigen-activated immune human peripheral blood mononuclear cells. *Infect Immun*. 1995;63:1047-1054.

6. Guedenon A, Zinsou C, Jose R, Andele K, Pritze S, Portaels F, Meyers WM. Traditional treatment of Buruli ulcer in Benin. *Arch Dermatol*. 1995:131:741-742.

7. Gormus BJ, Xu K, Baskin GB, Martin LN, Bohm RP Jr, Blanchard JL, Mack PA, Ratterree MS, McClure HM, Meyers WM, Walsh GP. Experimental leprosy in monkeys. II. Longitudinal serological observations in sooty mangabey monkeys. *Lepr Rev*. 1995;66:105-125.

8. Moran CA, Nelson AM, Tuur SM, Mputu L, Fonseca L, Meyers WM. Leprosy in five human immunodeficiency virus-infected patients. *Mod Pathol*. 1995;8:662-664.

9. Bosquee L, Bottger EC, DeBeenhouwer H, Fonteyne PA, Hirschel B, Larsson L, Meyers WM, Palomino JC, Realini L, Rigouts L, Silva MT, Teske A, Van der Auwera P, Portaels F. Cervical lymphadenitis caused by a fastidious mycobacterium closely related to *Mycobacterium*

genavense in an apparently immunocompetent woman: diagnosis by culture-free microbiological methods. *J Clin Microbiol*. 1995;33:2670-2674.

10. Meyers WM. Mycobacterial infections of the skin (including leprosy, tuberculosis of the skin, Buruli ulcer, and less common mycobacterial infections). In: Doerr W, Seifert G, eds. *Tropical Pathology*. 2nd ed. Berlin, Germany: Springer-Verlag; 1995:8:291-377.
11. Hadfield TL, Lamy Y, Wear DJ. Demonstration of *Chlamydia trachomatis* in inguinal lymphadenitis of lymphogranuloma venereum: a light microscopy, electron microscopy and polymerase chain reaction study. *Mod Pathol*. 1995;8:924-929.
12. Friedman HD, Hadfield TL, Lamy Y, Fritzinger D, Bonaventura M, Cynamon MT. Whipple's disease presenting as chronic wastage and abdominal lymphadenopathy. *Diagn Microbiol Infect Dis*. 1995;23:111-113.

DIVISION OF GEOGRAPHIC PATHOLOGY

Douglas J. Wear, M.D.
Acting Chief
Date of Appointment - 2 May 1991

MISSION

The division provided consultative services to military, Department of Veterans Affairs, and civilian hospitals in the USA and in many foreign countries. Several "regular contributors" in medical missionary hospitals in the Ubangi Territory of northern Zaire continued to submit specimens from cases of endemic tropical diseases of special research interest.

Fresh frozen biopsy or autopsy tissues, as well as blood specimens, from military and civilian hospitals were also submitted to the division. Numerous cases were also referred to the division for consultation from other departments of the AFIP.

STAFF

Medical

Aileen Marty, CDR, MC, USN, Chief, Infectious Disease Pathology Branch
(D) Yvonne L. Lamy, LTC, MC, USA, Staff Pathologist
Ronald Neafie, MS, Chief, Parasitic Disease Pathology Branch
(A, D) Freddie Lemons-Estes, CDR, MC, USN, Staff Pathologist

Scientific

(D) Ellen Andersen, LT, MSC, USNR, Chief, Parasitology Research Branch
(D) Leslie Williams, LTJG, MSC, USNR

CONSULTATION

Cases

Description	*Reported*
Military	131
Federal(VA/PHS)	36
Civilian	507
Interdepartmental	1,609

RESEARCH

Research projects during the year included: study of the growth of cat-scratch disease (CSD) bacillus from lymph nodes, blood, spleen, and spinal fluid; characterization of the CSD organism and its molecular biology; filarial disease; ehrlichiosis; and neural changes in primates with leprosy.

EDUCATION

The division pursued its educational mission by: (1) participating in a daily departmental slide conference for review of unique, controversial, difficult, or interesting cases; (2) conducting a program for international fellows and trainees; and (3) lecturing at the AFIP weekly staff conferences. Members of the division presented lectures and seminars at local, regional, national, and international meetings throughout the year; some were major presentations of 2 to 3 hours' duration.

CDR Marty served as adjunct assistant professor at the Uniformed Services University of Health Sciences.

PRESENTATIONS

1. Feb. 16, 1995: Orlando, Florida, Armed Forces Institute of Pathology course, Controversies and Recent Advances in Surgical Pathology, "Cat Scratch Disease," presented by D. Wear, M.D.
2. Feb. 17, 1995: Orlando, Florida, Armed Forces Institute of Pathology course, Controversies and Recent Advances in Surgical Pathology, "Lymphogranuloma Venereum," presented by D. Wear, M.D.
3. Feb. 17, 1995: Orlando, Florida, Armed Forces Institute of Pathology course, Controversies and Recent Advances in Surgical Pathology, "Interesting and Controversial Cases of Infectious Disease," presented by D. Wear, M.D.
4. Feb. 22, 1995: Takoma Park, Md., Columbia Union College health class, "Communicable Diseases," presented by D. Wear, M.D.
5. Feb. 23, 1995: Washington, D.C., Walter Reed General Hospital Pathology Department, Great Lecture Series, "Grain Forming Organisms," presented by R. Neafie, M.S.
6. Feb. 27, 1995: Washington, D.C., Walter Reed General Hospital Pathology Department, "Cat Scratch Disease/Bacillary Angiomatosis," presented by D. Wear, M.D.
7. Mar. 14, 1995: Toronto, Canada, annual meeting, International Academy of Pathology, Specialty Conference on Infectious and Inflammatory Disease, "Mycetomas," presented by R. Neafie, M.S.
8. Mar. 30, 1995: Washington, D.C., staff of the Department of Infectious and Parasitic Disease Pathology and students, "Tapeworms," presented by G. Cohen, M.D., Ph.D.
9. Apr. 11, 1995: Washington, D.C., annual course on Problems in Anatomic Pathology, "Infectious Disease Pathology: the 25 Unknowns," presented by R. Neafie, M.S.
10. May 17, 1995: Bethesda, Md., NIH, Unknown Slide Conference, presented by E. Marty, M.D.
12. May 31 to June 2, 1995: William Beaumont Army Medical Center, El Paso, Texas, Visiting Pathology Consultant Program, Department of Pathology, "Special Stains Useful in Identifying Microor-

ganisms," "Diagnosis of Bacterial Infections," "Diagnosis of Fungal Infection," "Diagnosis of Protozoal Infections," "Diagnosis of Helminthic Infections," "Case Presentations: 10 to 12 Unknowns," presented by R. Neafie, M.S.

13. July 24, 1995: Washington, D.C., Walter Reed Army Institute of Research, Tropical Medicine Course, "Case Presentations, Loiasis and Dirofilariasis, Parasitology Laboratory: Filariasis and Trypanosomiasis," presented by R. Neafie, M.S.
14. July 27, 1995: Washington, D.C., Walter Reed Army Institute of Research, Tropical Medicine Course, "Onchocerciasis," presented by E. Marty, M.D.
15. Sept. 11, 1995: Washington, D.C., "Histopathology of Gastrointestinal Infections: New Perspectives of Old and Newly Recognized Agents," presented by E. Marty, M.D.
16. Sept. 15, 1995: Washington, D.C., AFIP Senior Resident Program, "Leishmaniasis," presented by R. Neafie, M.S.
17. Oct. 11, 1995: Takoma Park, Md., Columbia Union College, "Sexually Transmitted Diseases," presented by D. Wear, M.D.
18. Oct. 1995: Hamburg, Germany, European Conference on Tropical Medicine, Symposium A2, 17:00, "Emerging and Reemerging Pathogens of the Gastrointestinal Tract in the Tropics," presented by E. Marty, M.D.
19. Nov. 1, 1995: Washington, D.C., AFIP Weekly Professional Staff Conference, "Three Unusual Cases," presented by R. Neafie, M.S.
20. Nov. 1, 1995: Washington, D.C., AFIP Weekly Professional Staff Conference, "Infectious Diseases of the Gastrointestinal Tract—Selected Cases," presented by A. Marty, M.D.
21. Nov. 1, 1995: Washington, D.C., AFIP Weekly Professional Staff Conference, "Plague," presented by D. Wear, M.D.
22. Nov. 30, 1995: Washington, D.C., National Museum of Health and Medicine, AFIP staff, "Overview of the Department and Infectious Diseases," presented by A. Marty, M.D.
23. Nov. 30, 1995: Washington, D.C., Museum staff, "The African Connection," presented by R. Neafie, M.S.
24. Nov. 30, 1995: Washington, D.C., Museum staff, "The Molecular Diagnosis of Infectious Diseases," presented by G. Cohen, M.D., Ph.D.
25. Nov. 30, 1995: Washington, D.C., Museum staff, "The Laboratory and Special Stains Used in Identifying Microorganisms," presented by Scott Denk.
26. Nov. 30, 1995: Washington, D.C., Museum staff, "Observation," presented by D. Wear, M.D.
27. Dec. 18, 1995: Washington, D.C., Walter Reed General Hospital Pathology Department, Guest Lecture Series, "Ten Unknowns," presented by R. Neafie, M.S.

PUBLICATIONS

Journal Articles

1. Hadfield TL, Lamy Y, Wear DJ. Demonstration of *Chlamydia trachomatis* in inguinal lymphadenitis of lymphogranuloma venereum: a light microscopy, electron microscopy and polymerase chain reaction study. *Mod Pathol.* 1995;8:924-929.
2. Marty AM, Dumler JS, Imes G, Brusman HP, Smrkovski LL, Frisman DM. Ehrlichiosis mimicking thrombotic thrombocytopenic purpura: case report and pathological correlation. *Hum Pathol*. 1995;26:920-925.
3. Tsai S, Wear DJ, Shin J W-K, Lo S-C. Mycoplasmas and oncogenesis: persistent infection and multistage malignant transformation. *Medical Sciences*. 1995;92:10197-10201.

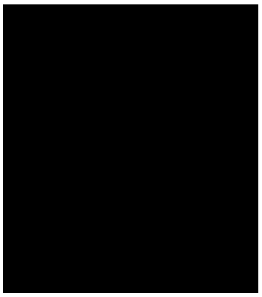
Books and Chapters

1. Zaki SR, Marty AM. New technology for the diagnosis of infectious disease. In: Doerr W, Seifert G, eds. *Tropical Pathology, VIII*. 2nd ed. Berlin, Germany: Springer-Verlag: 1995:4:127-154.

2. Marty AM, Andersen EM. Malaria. In: Doerr W, Seifert G, eds. *Tropical Pathology, VIII*. 2nd ed. Berlin, Germany: Springer-Verlag; 1995:13:557-596.
3. Marty AM, Andersen EM. Helminthology. In: Doerr W, Seifert G, eds. *Tropical Pathology, VIII*. 2nd ed. Berlin, Germany: Springer-Verlag; 1995;17:801-982.
4. Wear DJ, Lo S-C. Localization of mycoplasmas in tissues. In: Razin S, Tully J, eds. *Molecular and Diagnostic Procedures in Mycoplasmology*. San Diego, Calif: Academic Press; 1995;2:81-87.

Abstracts and Other Publications

1. Marty AM. Emerging and re-emerging pathogens of the gastrointestinal tract. In: European Conference on Tropical Medicine; October 22-26, 1995; Hamburg, Germany.



DIVISION OF MOLECULAR PATHOBIOLOGY

Shyh-Ching Lo, M.D., Ph.D.
Chief
Date of Appointment - 2 May 1991

STAFF

Medical

Shyh-Ching Lo, Ph.D., M.D., Division Chief

Scientific

Richard Wang, Ph.D., Senior Scientist (ARP)
Shien Tsai, Ph.D., Senior Scientist (ARP)
Shaw-Huey Feng, Ph.D., Molecular Biology Scientist (ARP)
Bin-Xue Zhang, Ph.D., M.D., Visiting Scientist (NIH Fellow)
Michael Hayes, M.S., Research Microbiologist (ARP)
Susan Ditty, B.A., Research Microbiologist (ARP)
Christine Davies, B.S., Molecular Biology Technician (ARP)
Bing-Jie Li, M.D., Molecular Biology Technician (ARP)
Jose Rodríguez, Research Technician (ARP)

MISSION

To perform basic research related to the pathogenesis of human diseases; to develop new techniques and new assays for detection of infection and diagnosis of various diseases; to refine existing diagnostic tools and techniques; to explore new areas of molecular biology and information that may be useful in understanding human disease process; and to consult with other CAP departments on the knowledge of various disease processes and the molecular techniques in diagnosis and research.

CONSULTATION

Consult on the histopathological diagnosis of mycoplasmal infections in patients with AIDS and non-AIDS patients with an acute fulminant disease. Consult on the disease process of mycoplasmal infections in various human diseases, including AIDS.

RESEARCH

In the past 2 years, the following studies have been carried out using three species of *Mycoplasma—Mycoplasma fermentans* (incognitus strain), *M penetrans,* or *M genitalium*. *M fermentans* is potentially associated with AIDS and adult respiratory distress syndrome. *M penetrans* is a new species isolated from AIDS patients and may be associated with Kaposi's sarcoma. Both *M fermentans* and *M penetrans* are shown to have oncogenic potential. *M genitalium* is likely a newly proven sexually transmitted disease. The results are briefly summarized as follows:

1. **Induced Mouse Spleen B-Cell Proliferation and Secretion of Immunoglobulin by Lipid-Associated Membrane Proteins (LAMPs) of *Mycoplasma fermentans* (*incognitus* strain) and *M penetrans*.** A group of lipid-associated membrane proteins (LAMPs) extracted by Triton X-114 from mycoplasmas are major antigenic targets of a human host's antibody responses. LAMPs prepared from both *M fermentans* and *M penetrans* stimulated nonspecifically spleen cells of CBA/CaH mice to proliferate. LAMPs also stimulated spleen cells from athymic mice. On the other hand, enriched splenic T cells from CBA/CaH mice with or without accessory cells responded poorly. Thus, the mitogenic effect of mycoplasmal LAMPs appeared mainly on B cells. High levels of immunoglobulin (Ig)M and low but detectable amounts of IgG were found in the supernatant of LAMPs-treated splenic cell culture. *M penetrans* LAMPs had a much more potent effect on murine spleen cells than *M fermentans* (*incognitus* strain) LAMPs, both in inducing B-cell proliferation and Ig secretion. In conclusion, the mycoplasmal LAMPs contained an active component(s) with T-independent B-cell mitogenic effect.

2. **Potent Activities of Cytokines and Nitric Oxide Induction by LAMPs from *Mycoplasma penetrans* and *Mycoplasma fermentans*.** LAMPs of *Mycoplasma penetrans* (MPe LAMPs) and *Mycoplasma fermentans* incognitus (MFi LAMPs) possess B-cell mitogenic activity in mice with potency and kinetics similar to another well-known B-cell mitogen, lipopolysaccharide (LPS). LPS is also a well-known cytokine and nitric oxide (NO) inducer. In peritoneal macrophages, both MPe and MFi LAMPs have a similar capacity as LPS in upregulating interleukin (IL)-1 Beta and tumor necrosis factor (TNF)-alpha mRNA production and inducing de novo synthesis of IL-6 and IL-12 p35 and p40 mRNA within 2 hours of stimulation. Large quantities of IL-6 and TNF-alpha proteins were secreted only by stimulated macrophages. IL-1 Beta produced was found to be entirely cell associated. Mycoplasmal LAMPs could also induce spleen cells to secrete IL-6 and TNF-alpha. In addition, IL-10 and IFN-gamma, which were not produced by stimulated peritoneal macrophages, were induced by stimulated spleen cells. Production of IFN-gamma was apparently regulated by factor(s) released by either LPS- or LAMP-stimulated macrophages. IL-10 appeared to be induced by a different mechanism in spleen. Mpe or MFi LAMPs became potent inducers of NO by macrophages in the presence of IFN-gamma (1 U/ml). Therefore, LAMPs of MPe and MFi contain many LPS-like activities, both as a B-cell mitogen and a cytokine inducer.

3. **Seroconversion Following *Mycoplasma penetrans* Infection in Patients with AIDS.** All six clinical isolates of *Mycoplasma penetrans* share extensive antigenic similarity and DNA homology. In serial serum samples from five of these six patients, four patients seroconverted and produced prominent antibodies to *M penetrans* at the time of mycoplasma isolation as measured by ELISA and Western blot (WB) using LAMPs as antigens. However, one mycoplasma-infected patient failed to mount a detectable response. The positive antibody reactivity was mainly directed against two major LAMPs with a molecular mass of 35 and 38 kDal. Documentation of seroconversion in serial serum samples from patients known to be infected with *M penetrans* further validates our serological assays for mycoplasma-specific antibodies using LAMPs.

4. **Serological Analysis of the Major Antigenic LAMPs in Six Clinical Isolates of *Mycoplasma penetrans*.** LAMPs are highly mycoplasmal species specific and are the primary antigenic

targets for host antibody response. However, variation of these mycoplasmal surface antigens is often found in vitro using Western blotting (WB) and monoclonal antibodies raised against LAMPs. These proteins may be different in size and have a distinct phase of expression, producing a wide spectrum of surface phenotypic diversity. This is proposed as an important mechanism that enables the organisms to evade host immune responses. The heterogeneity of LAMPs would challenge the suitability or adequacy of using LAMPs from a particular isolate for serological assays of the presence of mycoplasma-specific antibodies in infected patients. Antigenic profiles of LAMPs from six clinical isolates of *Mycoplasma penetrans* were studied using SDS-PAGE and WB with rabbit hyperimmune sera, monoclonal antibodies, and patients' sera that reacted positively with the *M penetrans* (GTU54-6A1) LAMPs. Differences in WB banding pattern and immune reactivity among different isolates were found with rabbit hyperimmune sera and mouse monoclonal antibodies. However, there was no detectable difference in immune reactivity against the six different clinical isolates when serum samples from the same six patients were tested. Thus, LAMPs from GTU-54 strain provide sufficient specific antigenic determinants and can be used to effectively detect antibodies in patients clinically infected by *M penetrans.*

5. ***Mycoplasma penetrans* Infection in Male Homosexuals with AIDS: High Seroprevalence and Association with Kaposi's Sarcoma.** An unusually high frequency of antibodies to *Mycoplasma penetrans* was found in male homosexuals with AIDS (55 of 149, 37%) and in HIV-infected asymptomatic homosexuals (13 of 49, 26.5%), but not in intravenous drug users (3 of 308, 0.9%) and hemophiliacs (1 of 165, 0.6%) with or without HIV-1 infection. Thus, both *M penetrans* and Kaposi's sarcoma (KS) occur primarily in male homosexuals, but rarely in other groups of patients at high risk of AIDS. Among 414 HIV-1 infected patients, statistical analysis revealed those with *M penetrans* antibody were 11.7 times more likely to develop KS. Furthermore, among 198 HIV-infected homosexuals (149 with AIDS and 49 without AIDS), those with KS had a significantly higher frequency of *M penetrans*-specific antibody (28 of 47, 59.6%) as compared to those without KS (27 of 102, 26.5%) with AIDS, as well as 13 of 49 without AIDS (also 26.5%)—odds ratio 4.1, $p < 0.001$. *M penetrans* is apparently transmitted sexually through homosexual activity and is epidemiologically linked to the formation of KS in gay men with AIDS. A parallel test with *M genitalium* revealed no similar link to KS in the same study sample.

6. **Cloning and Sequencing *Mycoplasma penetrans*' Gene Coding an Immunodominant LAMP Recognized by Sera of Patients with AIDS.** We identified the complete gene encoding the P38 KDal LAMP (see paragraph 3) by constructing the genomic expression library of *M penetrans* in lambda gt11 system and the genomic DNA library in lambda GEM-11. The predicted protein sequence revealed that the N-terminal portion of P38 contained a typical prokaryotic signal peptide sequence of 30 amino acids terminating with the tetrapeptide V-S-S-C, a characteristic motif recognized by prokaryotic prolipoprotein signal peptidase. The same 200-bp nucleotide sequence containing the 5' regulatory region and the sequence coding for the signal peptide, as well as the first six amino acids of the P38, was also found in two other *M penetrans* LAMPs genes.

7. **Identification of a Putative *infC-rpmI-rplT* Operon Flanked by Long Inverted Repeats in *Mycoplasma fermentans* (*incognitus* strain).** A specific 1542-bp DNA fragment was amplified from *Mycoplasma fermentans* (incognitus strain) using a unique 23-nucleotide (nt) synthetic deoxyribonucleotide (oligo) (5'-TCCAAAAAGTCCGGAATTTGGGG) as the primer pair in the polymerase chain reaction (PCR) (Hu, et al. *Gene*. 1990;93: 67-72). The amplified DNA was cloned and sequenced. There are four potential open reading frames (ORFs), which are arranged adjacent to each other with parts of ORF-1 and ORF-2 overlapping. The deduced amino acid (aa) sequences of ORF-2, ORF-3, and ORF-4 are 34% to 60% identical to the translation initiation factor IF3 (*infC* gene), ribosomal proteins L35 (*rpmI* gene) and L20 (*rplT* gene) of *Escherichia coli* and *Bacillus stearothermophilus*, respectively. There are multiple sites with

promoterlike sequences identified upstream of the putative *infC* gene in the mycoplasma. The cluster of genes (ORF-2, ORF-3, and ORF-4) is identified as a putative mycoplasma *infC-rpmI-rplT* operon. Most interestingly, our study reveals that this operon potentially constitutes a part of a mobile genetic element in the incognitus strain of *M fermentans*.

8. **Occurrence of High-frequency of DNA Rearrangements in the Chromosome of *Mycoplasma fermentans* Correlates with IS-like Element.** *Mycoplasma fermentans* is potentially associated with AIDS and adult respiratory distress syndrome. Two strains, M64 and SK6, isolated from patients with resporatory disease had deletion or duplication of the previously reported IS-like element at high frequency. These events may imply that the chromosome undergoes DNA rearrangements mediated by an IS-like element.

9. **Malignant Transformation of Mammalian Cells Mediated by Persistent Parasitic Infection by Mycoplasmas.** Oncogenic potentials of *Mycoplasma fermentans* and *M penetrans* were studied using a model culture system of C_3H 10T1/2 (C_3H) mouse embryo cells. Instead of acute transformation, a clear multistage process in promotion and progression of malignant cell transformation with long latency was observed to be mediated by these mycoplasmas. After six passages (1 wk/passage) of persistent infection with *M fermentans*, C_3H cells exhibited phenotypic changes with malignant characteristics that became progressively more prominent with further prolonged infection. However, up to at least the 11th passage all malignant changes were reversible if mycoplasmas were eradicated from cultures by treatment with antibiotics. In contrast, further persistent infection with the mycoplasmas until 18 passages resulted in an irreversible form of transformation that included high soft agar cloning efficiency and an ability to form tumors in animals. Onset of the irreversible phase of transformation coincided with marked chromosomal losses and changes in C_3H cells infected by either *M fermentans* or *M penetrans* (reversible stage less prominent). Genetic instability in the transformed cells was detected as marked chromosomal alterations, most likely caused by mutation of gene(s) responsible for fidelity of DNA replication or repairs. Once induced, these chromosomal alterations continued to evolve both in cultures and in animals without the continued presence of the transforming microbes. The mycoplasma-mediated multistage oncogenesis exhibited many characteristics found in natural neoplastic process in humans.

10. **Overexpression of H-ras and c-myc Oncogenes in Mycoplasma-Induced Cells Transformation.** H-ras and c-myc oncogenes, but not N-myc, src, N-ras, or P53 genes, were found highly activated during the mycoplasma-dependent stage of cell transformation. Following *M fermentans* eradication, H-ras and c-myc messengers quickly dissipated, coinciding closely with reversion of morphologic transformation. After 7 or 11 weeks of persistent *M penetrans*-infection, H-ras, but not c-myc, gene was also highly activated, but these C3H cells exhibited no morphological changes. Thus, activation of H-ras alone was not sufficient to effect transformation in C3H cells. After 18 weeks of persistent infection with *M fermentans* or *M penetrans,* both H-ras and c-myc oncogenes became constitutively activated and resulted in a permanent form of malignant transformation of C3H cells. This mycoplasma-mediated oncogenesis provides a unique model system to explore molecular mechanisms responsible for each stage in progressive malignant transformation.

11. **Pathogenicity of *Mycoplasma fermentans* and *Mycoplasma penetrans* in Experimentally Infected Chicken Embryos.** In an attempt to develop a laboratory model for studying pathogenesis of AIDS-associated mycoplasmas, embryonated chicken eggs were infected via the yolk sac with *Mycoplasma fermentans* or *Mycoplasma penetrans*. Embryo mortality was 10% to 30% for *M fermentans* infection and 70% to 100% for *M penetrans* infection at 10 days postinfection, immediately prior to hatching of the eggs. Morbidity of infected embryos included dwarfing, deformed limbs, and petechia. Both *M fermentans* and *M penetrans* were reisolated from allantoic fluid, yolk sac, liver, and brain of the embryos. There were apparent different histopathological changes in embryos dying of infections by the two mycoplasmas. The most prominent changes occurred in the livers. An acute inflammatory response with periportal

infiltration of granulocytes was normally found in *M penetrans* infection, while diffuse infiltration of lymphocytes was noted in *M fermentans* infection. This experimental system may provide a rare opportunity to study various mycoplasma-associated disease processes in animal tissues.

12. **Identification of a Previously Unrecognized Epidemic of Sexually Transmitted *Mycoplasma genitalium* Infection.** *Mycoplasma genitalium* is a fastidious Mollicute originally isolated from the urethra of two homosexual men with nongonococcal urethritis. The prevalence of *M genitalium* infection in humans is not known due to the difficulties of culture isolation from various clinical specimens. The mode of transmission has not been documented either. We have studied 1,333 serum samples from various patient groups for *M genitalium*-specific antibodies by ELISA using LAMPs as antigens. Antibodies to LAMPs from *M genitalium* show little cross reactivity to LAMPs from *M pneumoniae*. Antibodies to *M genitalium* were detected in 150 of 341 (44%) patients with AIDS and 41 of 127 (32.3%) HIV-infected asymptomatic patients. In contrast, only 21 of 381 (5.5%) HIV-negative healthy blood donors and 5 of 144 (3.5%) patients with malignant diseases tested positive. In this study, we found 126 of 340 (37.1%) HIV-negative patients attending STD clinics have positive antibodies. The positive antibody reactivity was mainly directed against six LAMPs of *M genitalium* as determined by Western blot. The results strongly suggest *M genitalium* is a sexually transmitted mycoplasma.

EDUCATION

Staff of the Molecular Pathobiology Division of the Department of Infectious and Parasitic Disease Pathology present novel information of infectious diseases to pathologists and infectious diseases specialists and scientists at AFIP and throughout the world through professional seminars and courses, national conferences such as the American Society for Microbiology (ASM) and the Interscience Conference on Antimicrobial Agents and Chemotherapy (ICAAC), as well as international conferences such as the International Organization for Mycoplasmology (IOM) and the International Conference on AIDS.

PRESENTATIONS

1. Invited panelist at the Specialty Conference on Infectious and Inflammatory Diseases, U.S.-Canadian Academy of Pathology, Toronto, Canada, March 1995. Presentation title: Acute Fatal Infection by *Mycoplasma fermentans*, presented by Dr. Shyh-Ching Lo.
2. Divisional lecturer, the 95th General Meeting of the American Society for Microbiology (ASM), Washington, D.C., May 1995. Session 218, Symposium title: The Immune Response to Mollicutes. Lecture title: Modulation of Immune Functions by Lipid-Associated Membrane Proteins of *Mycoplasma penetrans* and *Mycoplasma fermentans*: Stimulation of B-Cell Proliferation and Cytokine Production, presented by Dr. Shaw-Huey Feng.
3. Invited panelist, Paradigms in Cancer Biology and Cancer Therapy Symposium, Fairfax, Va., October 1995. Presentation title: Persistent Infection by Low-grade Microorganisms and Malignant Cell Transformation, presented by Dr. Shyh-Ching Lo.
4. Invited lecturer, the Symposium on Advances in Infections Due to Mycoplasmas in Humans, Center for Biomedical Investigation, Puebla City, Mexico, November 1994. Lecture title: *Mycoplasma fermentans* in AIDS and non-AIDS patients, presented by Dr. Shyh-Ching Lo.

PUBLICATIONS

1. Hayes MM, Foo H-H, Timenetsky J, Lo S-C. *In vitro* antibiotic susceptibility testing of different clinical strains of *Mycoplasma penetrans* isolated from patients with AIDS. *Antimicrob Agents Chemother*. 1995;39:1386-1387.
2. Wear DJ, Lo S-C. Localization of mycoplasmas in tissues. In: Razin S, Tully J, eds. *Molecular and Diagnostic Procedures in Mycoplasmology*. New York, NY: Academic Press 1995;1(sect A7).

3. Tsai S, Wear DJ, Shih J W-K. Lo S-C. Mycoplasmas and oncogenesis: persistent infection and multistage malignant transformation. *Proc Natl Acad Sci USA*. 1995;92:10197-10201.

Abstract

1. Hayes MM, Li B-J, Feng S-H. Infection of embryonated chicken eggs with *Mycoplasma fermentans* and *Mycoplasma penetrans*. In: Abstracts of the 95th General Meeting of the American Society for Microbiology. Washington, DC: American Society for Microbiology; 1995:303. Abstract G-35.
2. Timenetsky J, Pierce PF, Wang R Y-H, Macalalad A, Hayes MM. Isolation of mycoplasmas from rectal swabs of HIV+ and HIV- gay men. In: Abstracts of the 95th General Meeting of the American Society for Microbiology. Washington, DC: American Society for Microbiology; 1995:297. Abstract G-3.
3. Tsai S, Wear DJ, Lo S-C. Malignant transformation of mammalian cells mediated by chronic persistent infection of mycoplasmas: a hit and run process. In: Abstracts of the 95th General Meeting of the American Society for Microbiology. Washington, DC: American Society for Microbiology; 1995:304. Abstract G-39.
4. Wang R, Ditty S, Davies C, Grandinetti T, Shih J, Lo S-C. Cloning and sequencing *Mycoplasma penetrans'* gene coding an immunodominant lipid-associated membrane protein (LAMP) recognized by sera of patients with AIDS. In: Abstracts of the 95th General Meeting of the American Society for Microbiology. Washington, DC: American Society for Microbiology; 1995:304. Abstract G-41.
5. Zhang B, Shih J W-K, Tsai S, Lo S-C. Alteration of oncogenes expression in mammalian cells transformed by the AIDS-associated mycoplasmas. In: Abstracts of the 95th General Meeting of the American Society for Microbiology. Washington, DC: American Society for Microbiology; 1995:304. Abstract G-40.